Proposed Project: Chromatographic Separation and Comparative Metabolism of d- and l-Nicotine

Principal Investigator: John A. Thompson, Ph.D.
School of Pharmacy
University of Colorado

Co-Investigator: Dennis R. Petersen, Ph.D.

1. Present Research Related to Nicotine

In July 1979 we undertook a project (supported by The CTR) to develop analytical techniques for the accurate quantitation of nicotine and its major metabolites, cotinine and nicotine N-oxide, in mouse tissues at various times following a single intraperitoneal dose of nicotine. Furthermore, these techniques were to be applied to a determination of the pharmacokinetics (half-life and other kinetic parameters) of these compounds in males and females of three inbred strains of mice. The main goal of this work was to determine the effects of genotype and gender on the metabolism of nicotine in an animal model which is potentially important for future studies of the pharmacology of nicotine.

Analytical methods were successfully developed (a paper has been published and a second is in preparation) which were superior in many aspects to methods which had already been reported in the literature. Our pharmacokinetic studies of nicotine and metabolites in blood, liver, and brain of C57, DBA, and C3H mice will be complete by December 1982. This has been a very large project involving hundreds of mice in order to generate reliable kinetic data for three tissues from three strains of animals including both males and females. At the present time, the last of the experimental work is being completed and all of the calculations and plots are being prepared for publication and for our final report to The Council.

With the successful completion of the 3 1/2-year study outlined above, we have acquired a considerable amount of experience working with nicotine and its metabolites. At this time, we are very interested in pursuing additional research related to this important constituent of tobacco.

2. Outline of Plans for the New Project

It has recently been shown that the pharmacological effects of d-nicotine are qualitatively similar but quantitatively lower than those of the naturally occurring stereoisomer, l-nicotine (S. Ikushima et al., JPET 222:463, 1982). There is also evidence that l-nicotine in tobacco is partially racemized (converted to d-nicotine) during burning (H. Klus and H. Kuhn, Fachliche Mitteilungen der Austria Tabakwerke, 1, 1977). Smokers, therefore, may be exposed to both isomers of nicotine.

At the present time no good methods exist for the micro-scale separation and analyses of d- and l-nicotine in mixtures of the stereoisomers. The main goals of the proposed project include the development of appropriate analytical methods and comparisons of the rates of metabolism of d- and l-nicotine. This

information should be of considerable value to researchers who are involved in more detailed studies of the comparative pharmacology of d- and l-nicotine.

The specific goals are as follows:

a) Prepare recemic and pure d-nicotine by established methods.

b) Develop sensitive gas chromatographic and/or high performance liquid chromatographic techniques to separate and quantitate d- and l-nicotine. This work will involve the use of chiral chromatographic columns, chiral ion-pairs, and other techniques.

c) Attempt to extend the usefulness of the separation methods developed in b) to preparative scale work. This would greatly facilitate the preparation of the pure stereoisomers of nicotine from racemic mixtures.

d) Use the newly developed analytical methods to accurately determine the amount of racemization of l-nicotine which occurs during the burning of tobacco.

e) Measure the amount of d- and l-nicotine in tissues of mice exposed to tobacco smoke. This could be extended to analyses of blood samples from humans following smoking.

f) Investigate the relative rates of metabolism of d- and l-nicotine in animals (mice and rats) following administration of racemic nicotine (injections vs. smoke from d-nicotine-treated cigarettes).

g) Compare the effects of species, age, and gender on the metabolism of d- vs. l-nicotine.

h) Develop chromatographic methods for the separation of d- and l-nor-nicotine which could be used on a preparative scale. This would provide a source of the pure stereoisomers which could then be methylated (with ^{14}C- or ^{3}H-labeled methyl iodide) as an alternative source of enantiomerically pure radiolabeled d- and l-nicotine for pharmacological studies.

3. Duration of the Study

The duration depends on the results obtained during the first year. If the appropriate methods can be successfully developed, an additional two years would be required to adequately fulfill the goals outlined above.

4. Estimated Cost

a.	Personnel	
	1 full-time postdoctoral Research Associate	$17,000
	1 part-time laborat-ry assistant (animal care, lab maintenance, etc.)	6,000
	1 month of summer salary and benefits for P.I. and Co-Investigator	7,500
b.	Supplies and Chemicals: solvents, glassware, consumables, chromatographic columns, etc.	8,000
c.	Equipment maintenance and consumables for operation of instruments	4,000
d.	Animals	1,000
e.	Miscellaneous: office supplies, mail, travel, telephone, publications, etc.	2,000
	Total estimated budget (first year)	$45,500